INHIBITORY ACTION OF LACTIC ACID ON CERTAIN BACTERIA AND FUNGI *

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In his instructions for the cultivation of fungi and especially the isolation of pure strains, Duggar[1] recommends the use of lactic acid in the culture mediums. He claims that, in general, 0.5% of lactic acid is sufficient to prevent the growth of contaminating bacteria. As there is little or no available information concerning the inhibitory effects of lactic acid on any great variety of bacteria, although there is as to a few special forms, this work was undertaken with a view of determining the effect on common bacteria and certain fungi.

Apart from its bearing on methods of cultivation, there is possibly a more important relation between bacteria and lactic acid. Under pathologic conditions lactic acid is the most widely distributed and abundant acid in the body. It is found in the tissues, in the secretions of the stomach, in abscesses, and in various fluids of the body. It is asserted by Fischer that an increased production of acids in the tissues is responsible, to a large degree, for the occurrence of edema. Therefore, since lactic acid is so commonly found in the body, it would seem that the action of this acid on bacteria might be a truer test of the tolerance of bacteria toward acid, that is toward the effect of the hydrogen-ion, than would be that of an inorganic acid like hydrochloric, as we are more nearly approximating common pathologic conditions found in the body.

The method employed in these preliminary experiments was as follows:

Plain standard agar was prepared, Armour's peptone being used because of the inability to procure Witte's. This was carefully titrated so that after 1 hour of sterilization in the autoclave at 15 pounds' pressure it had a neutral reaction to phenolphthalein. Ten c.c. were placed in each tube. The lactic acid was used undiluted, 0.1 c.c. equaling 1% of lactic acid, and corresponding amounts for the varying percentages of acid. For the fungi and higher bacteria, a neutral 1% glucose agar was used.

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[1] Fungous Diseases of Plants, 1909.

The tubes of agar were melted and cooled to about 50 C., and the varying amounts of lactic acid added and mixed by careful rotating. When cooled to about 40 C. they were inoculated with 1 loopful of the organism from a 24-hour culture. This was mixed by rotating so that all the bacteria would come in equal contact with the acid and then slanted. Ordinarily 24 hours was sufficient for growth at 37 C., although in some cases with higher acid-content growth did not appear for 48 hours. Maximum growth with bacteria was generally attained in 48 hours. After 48 hours, the cultures were kept at room temperature for 7 days, but in no case was further growth appreciable. The higher bacteria and fungi generally required a longer time for development.

It was found that the addition of the lactic acid to the melted agar and the inoculation with the organisms while the medium was still liquid gave the best results, and also insured the constancy of the acid. The lactic acid-content of the medium was titrated with N/10 KOH, using the method of Harada,[2] and it was found that the amount of lactic acid as shown by neutralization was the same as had been added. However, if the acid were added to the unmelted agar and then boiled the lactic acid might be diminished.

In the present experiment, only 1 strain of each organism, except in the case of the streptococci to be mentioned later, was employed. Twenty-five different bacteria including most of the ordinary organisms were used, also 6 of the higher organisms.

TABLE 1

PERCENTAGE OF LACTIC ACID INHIBITING BACTERIAL AND FUNGOUS GROWTH

	%		%
B. diphtheriae	0.1	Streptococcus viridans	0.4
Spirillum Finkleri	0.2	B. Tuberculosis	0.5*
Spirillum Metchnikovii	0.2	B. lactis aerogenes	0.5
Sarcina lutea	0.2	B. enteritidis	0.6
B. typhosus	0.2	B. fecalis alcaligenes	0.6
B. dysenteriae	0.3	B. prodigiosus	0.9
B. cholerae-suis	0.3	B. pyocyaneus	0.9
B. paratyphosus A	0.3	B. violaceous	0.9
B. paratyphosus B	0.3	B. subtilis	1.2
Proteus vulgaris	0.3	B. mucosus	1.5
B. cloacae	0.3	Actinomyces bovis	2.3
Staphylococcus albus	0.3	Sporothrix schenckii	2.8
Staphylococcus citreus	0.3	Blastomyces	2.9
Staphylococcus aureus	0.4	Mucor mucedo	7.6
B. coli	0.4	Aspergillus flavus	8.6
B. rhinoscleromatis	0.4		

* Grown on glucose agar, 3%.

It was found that the majority of the organisms was inhibited with less than 1% of lactic acid. It is interesting to note that of the 2 organisms resisting 1% of acid, one was a spore-former, B. subtilis, and the other a capsulated organism, B. mucosus. The higher bacteria, including Actinomyces, Sporothrix, and Blastomyces were more than twice as resistant as the other bacteria, while the 2 fungi, Mucor mucedo and Aspergillus flavus, were decidedly tolerant, growing in a medium containing several per cent. of acid.

[2] Am. Jour. Med. Sc., 1916, 152, p. 243.

In Table 1 the figures represent the highest acid-content at which growth appeared, growth being totally inhibited with a greater acid-content. Growth, irrespective of its amount, is alone considered.

It was found on plotting a curve of the percentages of acid-toleration that the first portion of the curve which included the larger number of organisms tested was nearly a straight line, deviating only slightly from the perpendicular. There was a decidedly sharper curve in that portion which represented the acid resisted by the higher forms. However, it is probably true that if a sufficient number of the higher bacteria and fungi were tested the curve would approximate a straight line.

A series of tests were made using 12 different strains of streptococci, 5 being isolated from milk, the remainder from pathologic conditions. It was found that a wide range of tolerance to the acid existed in both groups, one organism being barely resistant, and some being among the most resistant of all the bacteria tested. However, when the average of these values was obtained, it was found not to be essentially different from what might have been expected of this type of organism as judged by other related bacteria. Harada[2] likewise found a varying tolerance of different strains of streptococci to lactic acid.

It is to be supposed that had different strains of other organisms obtained from a variety of sources been tested, a similar range of tolerance would have been found, but probably the average of these values would not have essentially affected the general form of the curve.

It was also found that related types of bacteria or those belonging to the same group, as for example, the staphylococci or the group containing B. typhosus, B. dysenteriae, and B. cholerae-suis, exhibited the same or only slightly varying tolerance. It is interesting to consider whether or not tolerance toward lactic acid, especially in pathogenic organisms, may not be as individual a characteristic as is the behavior of bacteria toward the various sugars.

The fact was brought out that the group of chromogenic bacilli, B. prodigiosus, B. pyocyaneus, and B. violaceus, which were all quite resistant to the acid, exhibited a progressively increasing amount of chromogenesis in direct proportion to the amount of acid present up to the limits of toleration. Jordan[3] in his work with B. pyocyaneus found that the presence of lactate in the medium increased pigment-

[3] Jour. Exper. Med., 1899, 4, p. 627.

production. It is undoubtedly due to the negative radical of the lactic acid rather than to the hydrogen-ion that the increasing pigment is produced. It has not apparently been noted heretofore that the presence of lactic acid or any of its salts has the same effect on B. prodigiosus, B. violaceus, or other chromogens.

SUMMARY

Lactic acid has a varying degree of inhibitory action on the various bacteria and fungi, the action being apparently less on the more complex forms than on the simpler bacteria.

In the majority of cases, 24 of 31 organisms, growth was inhibited with less than 1% of lactic acid.

Twelve strains of streptococci from a variety of sources, including milk and different pathologic conditions, were tested, and gave a wide range of tolerance; but the average of these values was 0.6%.

With chromogenic bacteria, pigment-production increased progressively up to the limits of tolerance.